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Hematological, biochemical reference intervals and morphological blood cell characterization of *Crocodylus acutus* in Colombia

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Abstract

The American crocodile (*Crocodylus acutus*) is a vulnerable species whose health assessment in Colombia is limited by important gaps in baseline hematological, biochemical, and cellular morphology information. This study established the first hematological and serum biochemical reference intervals and provides the first detailed description of peripheral blood cell morphology for captive female *C. acutus* in Colombia. Blood samples were collected from 42 clinically healthy captive females maintained under professional husbandry conditions. Heterophils were the predominant leukocyte population (56.3%), followed by lymphocytes (28.7%) and basophils (11.4%). Serum cholesterol (422.8 ± 63.7 mg/dL) exhibited the highest mean concentrations, whereas creatinine (0.3 ± 0.3 mg/dL) and blood urea nitrogen (3.3 ± 1.5 mg/dL) were the lowest. Peripheral blood consisted of erythrocytes, polychromatophils, thrombocytes, heterophils, eosinophils, basophils, azurophil-like cells, monocytes, and lymphocytes. Morphometric analyses showed that monocytes and heterophils were the largest leukocytes, whereas lymphocytes and basophils were the smallest. These findings provide the first comprehensive hematological, biochemical, and cellular morphology baseline data for captive female *C. acutus* in Colombia and constitute an important tool for veterinary diagnosis, conservation management, health monitoring, reintroduction programs, and future ecotoxicological studies.

Introduction

The American crocodile (*Crocodylus acutus*) is a widely distributed crocodilian species that occurs in 18 countries throughout its geographic range (Thorbjarnarson, 2010). Despite its broad distribution, the species is classified as Vulnerable by the IUCN (Rainwater et al., 2022). In Colombia, it was previously considered Critically Endangered (CR) due to reduced population size (Castaño-Mora, 2002) but following the discovery of new populations and the recovery of others, it is currently classified as Endangered (EN) (Morales-Betancourt et al., 2015). The species is also included in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), except for populations in Cuba, Mexico, and one population in Colombia, which are listed in Appendix II (CITES, 2023).

Most studies conducted in Colombia have focused on the ecology and population status of this species (Ulloa-Delgado, 2011; Viloria-Lagares et al., 2017; Guerra-Cárdenas et al., 2020). Although these studies are fundamental for conservation, important knowledge gaps remain that limit the implementation of effective conservation strategies. In particular, there are currently no studies in Colombia focused on the health status of *C. acutus*, despite its importance for the success of reintroduction programs, the development of conservation strategies, and immunological or environmental ecotoxicology studies (Davis et al., 2008; Dzul-Caamal et al., 2016).

Currently, veterinary diagnostics for crocodilians largely rely on visual assessments, descriptive analyses, or post-mortem examinations (Barajas-Valero et al., 2021; Balaguera-Reina et al., 2022). However, these approaches are often impractical because crocodiles rarely exhibit early signs of stress, distress, or disease (Lovely et al., 2007). Alternatively, more proactive approaches, such as blood analyses, have proven effective for assessing health status (Barajas-Valero et al., 2021). However, reference data, including hematological, cytological, and biochemical reference intervals, which are essential for health assessments (Maceda-Veiga et al., 2015) are limited (Balaguera-Reina et al., 2022) or nonexistent for *C. acutus* in Colombia. Therefore, the objective of this study was to establish hematological and serum biochemical reference intervals and describe the cellular morphology of

peripheral blood in *C. acutus*. By providing baseline health information, this study aims to strengthen current conservation and reintroduction programs.

Methods

Blood samples were collected from 42 clinically healthy captive female *C. acutus* maintained under professional husbandry conditions at the Nelly Sierra & CIA conservation breeding facility, located in Carmen de Apicalá, Tolima, Colombia. Only individuals that had not received medical treatment or undergone any veterinary intervention within the previous three months were included in the study (Barajas-Valero et al., 2021). Animals were restrained using capture poles and ropes, with the eyes covered to reduce stress and the jaws securely restrained (Vliet, 2014). Approximately 5 mL of blood was collected from the ventral coccygeal vein using a 20 or 21 G, 1.5-inch syringe. The volume of blood collected never exceeded 1% of the individual's body mass.

Immediately after collection, three fresh blood smears were prepared. Smears were air-dried at room temperature, fixed with absolute methanol and stained using Wright stain. Whole blood was transferred to EDTA tubes, gently mixed by inversion, stored at 4°C, and processed within 24 h of collection. Blood intended for serum biochemistry was placed into gel separator tubes, centrifuged at 4,000 rpm for 10 minutes, and the serum was stored at -20°C until analysis. Erythrocyte (RBC) and total leukocyte (WBC) counts were determined manually using a Neubauer hemocytometer following a 1:100 dilution in Natt and Herrick's solution. Differential leukocyte counts were performed manually by counting 100 leukocytes on stained blood smears under 100× magnification.

PCV was determined by the microhematocrit method after centrifugation at 12,000 rpm for 5 min. Total solids (TS) were measured using a handheld refractometer. Hemoglobin concentration (Hb) was determined by spectrophotometry using a BTS-350 analyzer (Biosystems, Barcelona, Spain). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated following the equations described by Eatwell et al. (2014).

Serum biochemical analyses were performed by spectrophotometry using a BTS-350 analyzer (Biosystems, Barcelona, Spain) with commercial reagent kits (Spinreact, Girona, Spain). The analytes measured included albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), cholesterol, triglycerides, blood urea nitrogen (BUN), uric acid (UA), creatinine (CREA), and creatine kinase (CK).

Blood cell morphology was evaluated from Wright-Giemsa stained blood smears using an Olympus DP27 digital camera coupled to an Olympus microscope under 100× magnification. Images were acquired using cellSens version 1.13 software (Olympus Corporation, Tokyo, Japan). Representative mature erythrocytes (>100 for every type of blood cell), polychromatophils, thrombocytes, heterophils, eosinophils, basophils, azurophils, monocytes, and lymphocytes were selected for morphometric analysis. Measurements were obtained using Fiji (ImageJ, National Institutes of Health, Bethesda, MD, USA) and included cell area, perimeter, major axis, minor axis, aspect ratio, and roundness.

Prior to reference interval estimation, data were assessed for normality using the Shapiro–Wilk test. Outliers were identified and removed according to the ASVCP guidelines before calculating the reference intervals. Reference intervals were estimated using the robust method. Lower and upper reference limits were calculated together with their corresponding 90% confidence intervals. In addition, the mean, standard deviation (SD), minimum and maximum values, sample size (n), and *P*-values from the Shapiro–Wilk normality test were calculated for each analyte. Morphometric measurements are presented as mean ± SD.

Results

Forty-two captive female *C. acutus* were sampled. Total length ranged from 105.5 to 198.5 cm (mean ± SD = 143.2 ± 27.4 cm). Hematological (Table 1) and serum biochemical (Table 2) reference intervals were established after the removal of statistical outliers. Sample sizes varied among analytes because some measurements were unavailable or could not be reliably quantified in all individuals, particularly creatinine (Creat) and uric acid (UA).

Hematological reference intervals were calculated for 40–42 individuals depending on the analyte. Hematocrit ranged from 17.0 to 29.0%, hemoglobin from 3.3 to 8.6 g/dL, and total leukocyte counts from 1.15 to 4.44 × 10^3 cells/µL. Heterophils were the predominant leukocyte population (mean = 56.3%), followed by lymphocytes (28.7%), basophils (11.4%), eosinophils (3.1%), and monocytes (0.4%).

Biochemical reference intervals were established using 28–41 samples depending on analyte availability. Serum cholesterol and glucose exhibited the highest mean concentrations (422.8 and 208.3 mg/dL, respectively), whereas creatinine and blood urea nitrogen presented the lowest values (0.3 and 3.3 mg/dL, respectively). Creatine kinase showed the greatest individual variability among all biochemical analytes (mean ± SD = 500.3 ± 284.1 U/L), while albumin exhibited comparatively low variation.

Table 1. Hematological reference intervals for clinically healthy captive female *Crocodylus acutus*. Mean, standard deviation (SD), lower (LL) and upper (UL) reference limits with corresponding 90% confidence intervals (CI), minimum and maximum observed values, sample size (n), and normality test *P*-values are presented for each analyte. Hematological analytes include hematocrit (HTO), plasma total protein (PPT), erythrocyte count (RBC), hemoglobin concentration (HB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leukocyte count (WBC), and differential leukocyte counts expressed as percentages of heterophils (H), lymphocytes (L), eosinophils (E), monocytes (M), and basophils (B).

Analyte	Mean	SD	LL (90%CI)	UL (90%CI)	Min	Max	n	P-value
PCV (%)	25.3	2.7	20.6 (19.18- 22.06)	30.3 (29.22-31.12)	17.0	29.0	40	0.0148
TS (g/dl)	8.2	1.2	5.8 (5.25-6.34)	10.7 (10.10-11.20)	5.4	10.8	42	0.0637
RBC (10^6/µl)	1.1	0.2	0.6 (0.48-0.70)	1.6 (1.45-1.67)	0.6	1.8	41	0.0951
HB (g/dl)	5.8	1.0	3.7 (3.31-4.22)	7.8 (7.42-8.37)	3.3	8.6	42	0.2446
MCV (fl)	241.2	37.8	167.0 (150-184)	315.4 (299-332)	161.1	345.3	41	0.1236
MCHC (g/dl)	23.1	3.4	17.1 (15.76-19.31)	28.8 (25.47-30.72)	15.9	31.3	42	<0.01
MCH (pg)	55.1	8.7	37.9 (34.10-41.90)	72.3 (68.4-76.20)	39.7	80.2	41	0.2891
WBC (10^3/µl)	3.1	0.8	1.5 (1.09-1.84)	4.8 (4.4-5.2)	1.15	4.44	42	0.1154
H (%)	56.3	8.8	38.9 (35.12-42.83)	73.7 (69.91-77.62)	38	74	42	0.711
L (%)	28.7	9.7	9.6 (5.39-13.91)	47.8 (43.65-52.14)	9	58	42	0.3503
E (%)	3.1	2.2	0 (0)	6.9 (5.79-8.16)	0	9	42	0.0107
M (%)	0.4	0.9	0 (0)	1.6 (0-2.46)	0	3	42	<0.01
B (%)	11.4	7.2	0 (0-0.26)	25 (21.71-27.60)	0	25	42	0.0333

Table 2. Serum biochemical reference intervals for clinically healthy captive female *Crocodylus acutus* from Colombia. Mean, standard deviation (SD), lower (LL) and upper (UL) reference limits with corresponding 90% confidence intervals (CI), minimum and maximum observed values, sample size (n), and normality test *P*-values are presented for each analyte. Biochemical analytes include creatinine (Creat), blood urea nitrogen (BUN), uric acid (UA), albumin (Alb), cholesterol (Col), triglycerides (Trig), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and creatine kinase (CK).

	Mean	SD	LL (90%CI)	UL (90%CI)	Min	Max	n	P-value
CREA (mg/dl)	0.3	0.3	0 (0-0.019)	0.4 (0.26-0.63)	0.01	1.37	28	<0.01
Bun (mg/dl)	3.3	1.5	0.6 (0.19-1.18)	5.8 (4.83-6.73)	1.27	6.93	35	0.0235
UA (mg/dl)	7.1	3.1	1.3 (0.39-2.51)	12.5 (10.31-14.41)	2.7	14.8	35	0.0232
ALB (gr/dl)	1.7	0.3	1.2 (1.02-1.38)	2.2 (2.11-2.27)	0.94	2.05	41	<0.01
CHOL (mg/dl)	422.8	63.7	297.8 (269.64-326.07)	547.7 (519.48-575.91)	283.5	523.8	41	0.0680
TRIG (mg/dl)	48.2	40.4	0 (0)	118.1 (90.87-140.86)	1.1	161.9	41	<0.01
ALP (u/l)	31.5	10.9	10.1 (5.21-15.01)	52.9 (48.06-57.86)	9.5	61.4	40	0.6338
AST (u/l)	99.5	22.1	56.1 (46.23-66.06)	142.8 (132.92-152.75)	63.9	150.3	40	0.0639
CK (u/l)	500.3	284.1	117.0 (87.11-157.24)	1497.8 (1114.88-2012.40)	86.6	1347	39	0.0761

Regarding cellular morphology, peripheral blood cells of *C. acutus* consisted of erythrocytes, polychromatophils, thrombocytes, heterophils, eosinophils, basophils, azurophils, monocytes, and lymphocytes (Fig. 1). Erythrocytes were the most abundant circulating blood cells. They were elliptical, with pale blue to pink cytoplasm under Wright staining and a centrally located, round nucleus containing condensed chromatin (Fig. 1A). Polychromatophils were less frequently observed than mature erythrocytes. These cells were more rounded, although they retained an elliptical shape, and were considerably larger than mature erythrocytes (Table 3). Their cytoplasm exhibited a staining pattern slightly more basophilic than that of mature erythrocyte, while their nuclei were proportionally larger, round, centrally located, and contained less condensed chromatin. Thrombocytes occurred individually or in small aggregates. Both resting and activated forms were observed, although activated thrombocytes were uncommon (Fig. 1C). Resting thrombocytes were elliptical, whereas activated thrombocytes were rounded. Both forms possessed scant, pale blue-gray to nearly colorless cytoplasm and a centrally located oval nucleus with moderately condensed chromatin.

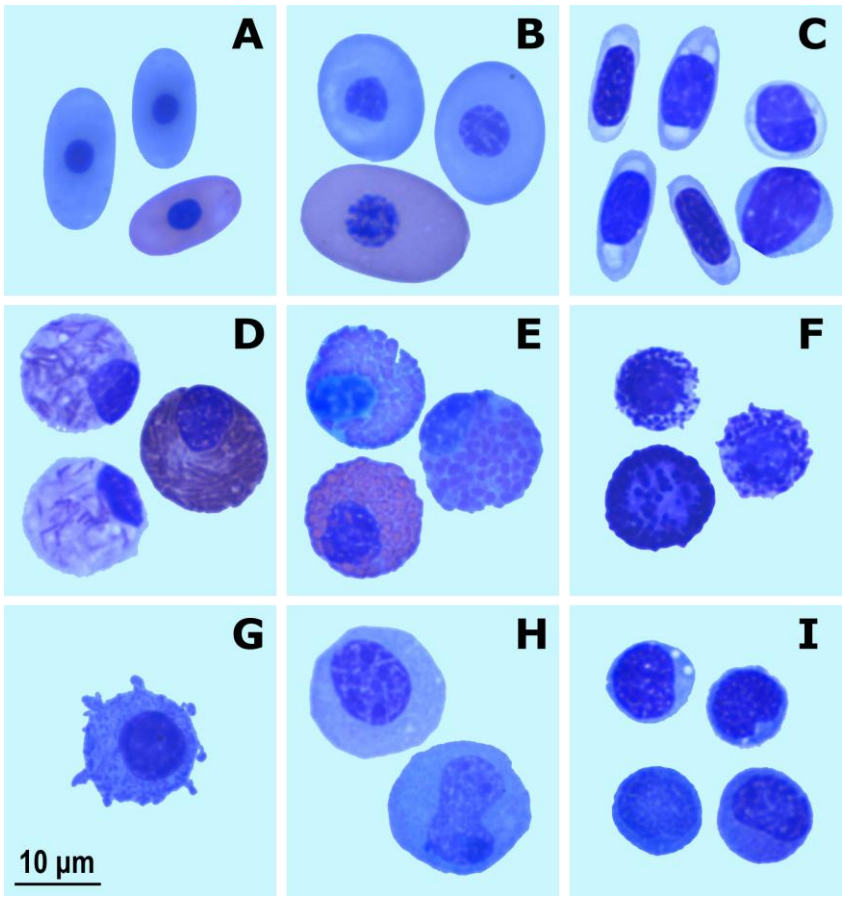


Figure 1. Representative photomicrographs of peripheral blood cells from clinically healthy captive female *Crocodylus acutus*. (A) Mature erythrocytes. (B) Polychromatophils. (C) Resting (left) and activated (right) thrombocytes. (D) Heterophil. (E) Eosinophil. (F) Basophil. (G) Azurophil-like cell. (H) Monocyte. (I) Lymphocyte. Blood smears were stained with Wright stain. Scale bar = 10 µm.

Heterophils were the predominant leukocyte. They were among the largest white blood cells and were round in shape, containing numerous elongated to fusiform, intensely eosinophilic granules that frequently overlapped and partially obscured the cytoplasm. The nucleus was eccentrically positioned, round to oval, and stained dark basophilic (Fig. 1D). Eosinophils were infrequently observed. These cells were spherical and contained abundant, large, rounded eosinophilic granules within a pale blue to colorless cytoplasm. The nucleus was generally eccentric, round to oval, and exhibited lighter basophilic staining than that of heterophils (Fig. 1E). Basophils were more common than eosinophils and appeared as spherical cells densely packed with coarse, intensely basophilic granules that frequently obscured both the cytoplasm and the nucleus. When visible, the nucleus was round and centrally located (Fig. 1F). Azurophil-like cells were rarely observed. These cells were round and contained abundant pale to blue-gray cytoplasm with fine azurophilic granules and, in some cases, short cytoplasmic projections or pseudopodia. The nucleus was centrally located, round to oval, and intensely basophilic (Fig. 1G).

Monocytes were among the largest circulating leukocytes and displayed abundant pale blue to purple cytoplasm. Occasional cytoplasmic vacuoles were observed. The nucleus was centrally located, oval to reniform (U-shaped in some cells), with loosely to moderately condensed basophilic chromatin (Fig. 1H). Lymphocytes were frequently observed and exhibited marked variation in cell size. They were round cells with a high nucleus-to-cytoplasm ratio. The round, dark basophilic nucleus occupied most of the cell and contained condensed chromatin, while the cytoplasm was scarce and stained a deep blue tone and occasionally exhibited slight pseudopodia (Fig. 1I).

Table 3. Morphometric measurements of peripheral blood cells from clinically healthy captive female *Crocodylus acutus*. Values are presented as mean ± standard deviation (SD). Cell area (μm²), perimeter (μm), major axis length (μm), minor axis length (μm), aspect ratio (major axis/minor axis), and roundness. Aspect ratio and roundness are dimensionless parameters.

Measure	Erythrocite	Polychromatophils	Thrombocytes	Heterophil	Eosinophil	Basophil	Monocyte	Lymphocyte
Area	89.41 ± 14.81	142.66 ± 22.61	58.86 ± 10.93	152.94 ± 31.02	132.78 ± 33.44	78.09 ± 18.79	151.7 ± 35.89	83.88 ± 14.1
Perimeter	36.53 ± 2.82	43.45 ± 3.45	31.51 ± 3.4	43.67 ± 4.52	40.56 ± 5.3	31.14 ± 3.92	43.43 ± 5.11	32.38 ± 2.87
Major axis	15.1 ± 1.21	16.25 ± 1.56	13.64 ± 1.85	14.2 ± 1.53	13.25 ± 1.76	10.15 ± 1.33	14.11 ± 1.69	10.54 ± 1.02
Minor axis	7.52 ± 0.8	11.16 ± 1.03	5.52 ± 0.87	13.6 ± 1.44	12.57 ± 1.67	9.67 ± 1.24	13.54 ± 1.63	10.07 ± 0.87
Aspect Ratio	2.02 ± 0.19	1.46 ± 0.16	2.53 ± 0.5	1.04 ± 0.06	1.06 ± 0.05	1.05 ± 0.07	1.04 ± 0.05	1.05 ± 0.05
Roundness	0.5 ± 0.05	0.69 ± 0.07	0.41 ± 0.11	0.96 ± 0.05	0.95 ± 0.05	0.95 ± 0.06	0.96 ± 0.05	0.96 ± 0.05

Morphometric measurements of peripheral blood cells are summarized in Table 3. Among all cell types, monocytes, heterophils, and polychromatophils exhibited the largest mean cell areas, whereas thrombocytes presented the smallest. Mature erythrocytes displayed an intermediate cell area but were characterized by an elongated shape, with an aspect ratio of 2.02 ± 0.19, while thrombocytes were the most elongated cells (aspect ratio = 2.53 ± 0.50). In contrast, all leukocyte types exhibited aspect ratios close to 1.0, indicating a predominantly rounded morphology. Roundness values were consistent with these observations, with heterophils, eosinophils, basophils, monocytes, and lymphocytes presenting values close to 1.0, whereas erythrocytes and thrombocytes had lower roundness values (0.50 ± 0.05 and 0.41 ± 0.11, respectively), reflecting their elliptical morphology.

Discussion

This study establishes the first hematological and serum biochemical reference intervals and provides the first detailed description of peripheral blood cell morphology for captive female *C. acutus* in Colombia. Although hematological and biochemical values may vary among populations (Stacy & Whitaker, 2000), these data provide an essential baseline for clinical diagnosis, health assessment, and disease monitoring in both captive and free-ranging animals (Stacy et al., 2011; Dervas et al., 2023; Lisičić et al., 2013).

The peripheral blood cell morphology of *C. acutus* was generally consistent with previous descriptions for crocodilians and other reptiles (Gutiérrez-Cervantes et al., 2021; Barajas-Valero et al., 2021). Mature erythrocytes displayed the characteristic elliptical shape and centrally located nucleus consistent with other reptile species (Stacy et al., 2011), whereas thrombocytes occurred individually or in small aggregates, with activated cells adopting a rounded morphology consistent with platelet activation. Polychromatophils were larger than mature erythrocytes, differing from the pattern reported for other reptiles (Stacy et al., 2011) but agreeing with the description for *C. intermedius* (Barajas-Valero et al., 2021).

Heterophils exhibited abundant fusiform eosinophilic granules and eccentrically positioned nuclei, matching the characteristic morphology described for other reptiles (Saint Girons, 1970; Montali, 1988). Likewise, eosinophils possessed large rounded eosinophilic granules and centrally to eccentrically positioned nuclei, features considered typical for reptiles (Montali, 1988; Campbell, 2004; Stacy et al., 2011). Basophils were among the smallest leukocytes, with dense basophilic granules frequently obscuring the nucleus, consistent with previous descriptions (Frye, 1991). Similarly, lymphocytes diameter or major axis measured 10.54 ± 1.02 μm, remaining within the expected 5–15 μm range for other species of mammals and birds (Sypek, 1988) with important size variations.

Monocytes exhibited abundant pale blue-gray cytoplasm, occasional cytoplasmic vacuoles, and oval to reniform nuclei, closely corresponding to the morphology reported for reptilian monocytes (Sypek, 1988). Azurophil-like cells were rarely observed in the present study, consistent with reports for captive *C. intermedius* (Barajas-Valero et al., 2021). Although azurophils are regarded as a characteristic leukocyte of reptiles and are commonly described (Montali, 1988; Alleman et al., 1999), their frequency varies among species (20). Thus, the low abundance observed in healthy captive *C. acutus* is likely a species-specific characteristic rather than an indication of disease.

The only crocodilian species for which a similarly comprehensive morphometric characterization of peripheral blood cells has been reported is *C. intermedius* (Barajas-Valero et al., 2021), and the overall morphometric pattern observed in *C. acutus* was largely consistent with that study. Most blood cell types exhibited comparable dimensions, with heterophils and monocytes representing the largest circulating leukocytes and lymphocytes and basophils among the smallest. Nevertheless, heterophils were smaller and lymphocytes slightly larger in *C. acutus*, whereas the greatest difference was observed in basophils, which were markedly smaller than those reported for *C. intermedius*. Although these values remain within the general size range reported for reptilian basophils (7–12 μm, occasionally reaching 20 μm) (Sypek, 1988), they suggest species-specific variation. Such differences may also reflect variation in age structure, physiological condition, or methodological factors including smear preparation, staining procedures, image acquisition, and measurement protocols (Hawkey et al., 1991; Salakij et al., 2022).

In addition to conventional morphometric measurements, this study quantified perimeter, aspect ratio, and roundness for all peripheral blood cell types. These shape descriptors complement size measurements by objectively characterizing cellular geometry and provide standardized metrics that may facilitate future comparative studies, automated image analysis, and the detection of subtle morphological changes associated with disease, physiological variation, or environmental stressors.

Variation among published hematological and biochemical values is expected because these parameters are influenced by numerous intrinsic and extrinsic factors, including species, sex, age, reproductive status, nutritional condition, environmental conditions, season, husbandry practices, and analytical methodology (Davis et al., 2008; Barajas-Valero et al., 2021). Consequently, direct comparisons among studies should be interpreted cautiously, particularly when comparing captive and free-ranging populations or studies performed using different laboratory techniques.

Within the erythrogram, hematocrit, erythrocyte count, and mean corpuscular volume were generally comparable to those reported for captive *C. acutus* and *C. intermedius* (Velit et al., 2020; Barajas-Valero et al., 2021), suggesting conservation of erythrogram parameters among crocodilians maintained under similar husbandry conditions. Total solids were higher than those reported for wild *C. acutus* and slightly exceeded values described for captive *C. rhombifer* and *C. intermedius* (Barajas-Valero et al., 2021; Balaguera-Reina et al., 2022; Palmer et al., 2023). This difference may reflect variation in age composition, reproductive physiology, high-protein captive diets, or methodological differences among studies. Previous studies in *C. niloticus* have also demonstrated significant age-related variation in PCV, hemoglobin, total protein, globulins, and AST (Lovely et al., 2007), highlighting the influence of ontogeny on hematological and biochemical values.

Greater variation was observed within the leukogram. Total leukocyte counts were substantially lower than those reported for wild *C. acutus* and lower than those described for captive *C. acutus*, *C. rhombifer*, and *C. intermedius* (Velit et al., 2020; Barajas-Valero et al., 2021; Palmer et al., 2023). Animals maintained under controlled captive environments are generally exposed to fewer pathogens, reduced intraspecific competition and physical aggression, and lower seasonal environmental stress than wild

populations. These conditions have been proposed to contribute to lower leukocyte counts in captive crocodilians (Barajas-Valero et al., 2021) and may partially explain the WBC values observed in this study. Heterophils were the predominant circulating leukocyte, accounting for more than 50% of the differential count, consistent with previous reports for captive *C. acutus* and *C. intermedius* (Velit et al., 2020; Barajas-Valero et al., 2021) and with the typical leukocyte profile described for crocodilians and chelonians (Mateo et al., 1984; Alleman et al., 1992; Christopher et al., 1999; Stacy et al., 2011). In contrast, wild *C. acutus* and captive *C. rhombifer* exhibited lymphocyte-dominated leukograms (Balaguera-Reina et al., 2022; Palmer et al., 2023). Relative leukocyte proportions are influenced by species, sex, season, physiological condition, and environmental factors (Saint Girons, 1970; Alleman et al., 1992), while manual differential counts introduce additional methodological variation (Stacy & Whitaker, 2000), therefore, comparisons among studies should be interpreted cautiously.

The serum biochemical profile likewise demonstrated both conserved and variable analytes among crocodilians. Albumin, creatinine, and uric acid were generally comparable to values reported for other crocodilian species, although mean uric acid concentrations were higher than those described for wild *C. acutus* and captive *C. intermedius* (Barajas-Valero et al., 2021; Balaguera-Reina et al., 2022). Unlike mammals, uric acid is the primary nitrogenous waste product in reptiles and therefore a more reliable indicator of renal function than creatinine (Frye, 1991; Eatwell, 2014). Despite being comparatively higher, the mean uric acid concentration remained within the normal physiological range for reptiles (<10 mg/dL) and well below values associated with renal disease (>15 mg/dL) (Thrall, 2012), suggesting normal physiological variation rather than impaired renal function.

Enzymatic activities also varied among studies. Creatine kinase activity was substantially lower than that reported for wild *C. acutus* and *C. johnstoni*, and captive *C. intermedius*, but comparable to captive *C. rhombifer* (Scheelings et al., 2016; Barajas-Valero et al., 2021; Balaguera-Reina et al., 2022; Palmer et al., 2023). Because CK is highly sensitive to skeletal muscle damage and physical exertion (Thrall, 2012), these differences most likely reflect variation in capture techniques, restraint duration, and handling procedures rather than intrinsic physiological differences. Likewise, differences in AST and alkaline phosphatase may be associated with age, growth, muscle activity, reproductive condition, hepatic metabolism, or analytical methodology.

The most notable biochemical difference was the markedly higher cholesterol concentration compared with other crocodilian species (Stacy & Whitaker, 2000; Padilla et al., 2011; Barajas-Valero et al., 2021). Cholesterol concentrations in reptiles are influenced by diet, nutritional status, sex, age, season, temperature, husbandry, and reproductive condition (Anderson et al., 2011; Adelakun et al., 2017; Sobrino-Yacobi et al., 2025). In *C. moreletti*, captive individuals also exhibit higher cholesterol concentrations than free-ranging populations (Padilla et al., 2011). Because this study included only clinically healthy captive females, reproductive physiology should also be considered. During vitellogenesis, cholesterol is mobilized for yolk formation, frequently increasing circulating cholesterol concentrations (Pearson, 2016). Therefore, the elevated cholesterol values observed likely reflect the combined effects of captive management, diet, nutritional status, and the possible reproductive condition of the sampled females rather than pathological alterations. Consequently, these reference intervals may not fully represent males, other age classes, or free-ranging populations, and seasonal or reproductive variation could not be evaluated. Future studies including a broader range of populations, demographic groups, and sampling periods will further refine these reference intervals and improve our understanding of physiological variation in *C. acutus*.

Overall, the present study provides a baseline for the clinical evaluation of captive female *C. acutus* in Colombia. These findings emphasize that hematological and biochemical values derived from other species or populations should not be directly extrapolated to Colombian *C. acutus*. Instead, population-specific reference intervals should be used whenever available to improve veterinary diagnosis, conservation management, reintroduction programs, and future health-monitoring and ecotoxicological studies.

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